REMARKS

A check for the fees for an extension of time and for filing an RCE is enclosed. Any fees that may be due in connection with this application may be charged to Deposit Account No. 50-1213. If a Petition for extension of time is needed, this paper is to be considered such Petition.

Claims 1-6, 9-34, 40-51, 54-61 and 63-72 and 78, 82-94 are pending in this application. Claims 73-77 and 79-81 are cancelled without prejudice or disclaimer. Claims 1, 5, 6, 25, 31, 40, 70, 76, 78, 87, 88 and 92 are amended to more particularly point out and distinctly claim the subject matter. Basis for some of the amendments, can be found, for example, at page 27, second paragraph and in Figure 8. Claim 70 also is amended by incorporating claim 75 therein, and claim 78 is rewritten as an independent claim. Therefore, no new matter is added.

THE REJECTIONS OF CLAIMS 1-6, 9-34, 40-51, 54-61 and 63-94 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

ENABLEMENT

Claims 1-6, 9-34, 40-51, 54-61 and 63-94 are rejected under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for capillaries dispensing with the range of 0.2-2 nL, allegedly does not provide enablement for the vesicle to dispense in the "sub to low" range. This rejection is respectfully traversed. It is respectfully submitted that claims 25-34, which do not recite "sub to low" are not within the purview of this rejection.

RELEVANT LAW

In order to satisfy the enablement requirement of 35 U.S.C §112, first paragraph, the specification must teach one of skill in the art to make and use the invention without undue experimentation. *Atlas Powder Co. v. E.I. DuPont de Nemours*, 750 F.2d 1569, 224 USPQ 409. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction

or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988).

ANALYSIS

It is respectfully submitted that the application clearly teaches those of skill in the art how to obtain and dispense an amount of material in the "sub to low" range. For example, the specification states, at p. 19, 2nd paragraph, that:

> The sample **arrays** are then analyzed by mass spectrometry to collect spectra data that is representative of the composition of the samples in the array. It is understood that the above methods provide processes that allow for rapidly dispensing definite and controlled volumes of analyte material. **In particular these processes allow for dispensing sub to low nanoliter volumes of fluid.** These low volume deposition techniques generate sample arrays well suited for analysis by mass spectrometry.

It is respectfully submitted that the specification teaches those of skill in the art how to dispense "sub to low" nanoliter volumes, other than 0.2-20 nl, onto a substrate without undue experimentation. It is clear that one of skill in the art could routinely increase the volume dispensed by merely increasing the number of droplets dispensed onto a substrate.

At page 22, the specification states:

> In the former, a 'piezoelectric pipette' (70 μm id capillary) dispenses single or multiple-0.2 nL droplets of matrix, and then analyte, onto the chip; spectra from as low as 0.2 fmol of a 36-mer DNA have been acquired using this procedure.

Hence, it is clear that single or multiple droplets can be dispensed rendering it routine to dispense any selected volume from 0.2 nl and up by adjusting the number of droplets.

On page 24 the specification states:

> The dispensed volume is controlled from 10^{-10} to 10^{-6} L by adjusting the number of droplets dispensed.

On page 28, the specification states:

> Sample delivery by the tool was examined using radio-labeled solutions and the phosphorimager as described previously; it was determined that each pin delivers approximately 1 nL of liquid. The spot-to-spot reproducibility is high.

Thus, it would be unfair and unduly limiting to require the claims to recite that the volume delivered is 0.2 nL to 20 nL, when it is clear from the specification that volumes outside this range also can be delivered by selecting an appropriate number of droplets.

WRITTEN DESCRIPTION

Claims 1-6, 9-34, 40-51, 54-61 and 63-94 are rejected under 35 U.S.C. 112, first paragraph, as allegedly failing to provide adequate written description because the claims now recite the limitation "such that spot-to-spot characteristics are reproducible in the array," and it is alleged that no such recitation was found in the specification. This rejection is respectfully traversed.

RELEVANT LAW

The purpose behind the written description requirement is to ensure that the patent applicant had possession of the claimed subject mater at the time of filing of the application. *In re Wertheim*, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). The manner in which the specification meets the requirement is not material; it may be met by either an express or an implicit disclosure.

35 U.S.C. §112 requires a written description of the invention. This requirement is distinct from and not coterminous with the enablement requirement:

> The purpose of the "written description" requirement is broader than to merely explain how to "make and use"; the applicant must also convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the "written description" inquiry, whatever is now claimed." *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563-64, 19 USPQ2d at 1117 (emphasis in original).

Accordingly, a specification must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the

invention, *i.e.*, whatever is now claimed. *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64, 19 USPQ.2d 1111, 1117 (Fed. Cir. 1991). A written description requirement issue generally involves the question of whether the subject matter of a claim is supported by or conforms to the disclosure of an application as filed. The test for sufficiency of support in a patent application is whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." *Ralston Purina Co. v. Far-Mar-Co., Inc.*, 772 F.2d 1570, 1575, 227 USPQ 177, 179 (Fed. Cir. 1985) (quoting In re Kaslow, 707 F.2d 1366, 1375, 217 USPQ 1089, 1096 (Fed. Cir. 1983)) (see also, MPEP 2163.02).

The Examiner has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in applicant's disclosure a description of the invention defined by the claims. *In re Wertheim*, 541 F.2d 257, 265, 191 USPQ 90, 98 (CCPA 1976).

ANALYSIS

Applicant respectfully disagrees with the Examiner's assertion, at page 2, last paragraph of the Office Action, that:

> Independent claims 1, 5, 25, 31, 40, 63, 66, and 70 now recite the limitation "such that spot-to-spot characteristics are reproducible in the array." No such recitation was found in the specification.

As set forth above, the specification, for example, at p. 19, 2nd paragraph, teaches that:

> The sample **arrays** are then analyzed by mass spectrometry to collect spectra data that is representative of the composition of the samples in the array. It is understood that the above methods provide processes that allow for rapidly dispensing definite and controlled volumes of analyte material. In particular these processes allow for dispensing sub to low nanoliter volumes of fluid. These low volume deposition techniques generate sample arrays well suited for analysis by mass spectrometry. For example, **the low volumes yield reproducibility of spot characteristics,**

In addition, the specification, at page 23, 2nd paragraph, teaches:

> Robot-driven serial and parallel pL-nL dispensing tools were used to generate 10-10^3 element DNA arrays on <1" square chips with flat or geometrically altered (e.g. with wells) surfaces for matrix assisted laser desorption ionization mass spectrometry analysis. In the former, a 'piezoelectric pipette' (70 μm id capillary) dispenses single or multiple-0.2 nL droplets of matrix, and then analyte, onto the chip; spectra from as low as 0.2 fmol of a 36-mer DNA have been acquired using this procedure. Despite the fast (<5 sec) evaporation, **micro-crystals of 3-hydroxypicolinic acid matrix containing the analyte are routinely produced resulting in higher reproducibility than routinely obtained with larger volume preparations**; all of 100 five fmol spots of a 23-mer in 800 μm wells yielded easily interpreted mass spectra, with 99/100 parent ion signals having signal to noise ration of >5.

In addition, the specification recites, on page 24, that:

> Sample delivery by the tool was examined using radio-labeled solutions and the phosphorimager as described previously; it was determined that each pin delivers approximately 1 nL of liquid. **The spot-to-spot reproducibility is high.**

As set forth, specification clearly teaches that the spot-to-spot reproducibility of the spot characteristics is high, and this results from the deposition of low volumes of sample and the delivery of defined and controlled volumes. Accordingly, it is respectfully submitted that those of skill in the art, in view of the specification, would clearly recognize that at the time of filing of the application, Applicant was in possession of a method of forming an array of spots of sample material on the substrate, "such that spot-to-spot characteristics are reproducible in the array" and that the specification clearly conveys this.

THE REJECTION OF CLAIMS 1-4 UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 1-4 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that applicant regards as the invention because the terms "sub" and "low" are relative terms that are alleged to render the claims indefinite. This rejection is respectfully traversed.

RELEVANT LAW

The purpose of 35 U.S.C. §112, second paragraph, is to permit those who would endeavor, in future enterprise, to approach the area circumscribed by the claims of a patent and to determine the metes and bound of protection so that they can evaluate the possibility of infringement with a reasonable degree of certainty. *In re Hammack*, 427 F.2d 1378, 166 USPQ 204 (CCPA 1970). When one skilled in the art would understand all of the language in the claims when read in light of the specification, a claim is not indefinite. *Rosemount Inc. v. Beckman Instruments, Inc.*, 727 F.2d 1540, 1547, 221 USPQ 1, 7 (Fed. Cir. 1984), *Caterpillar Tractor Co. v. Berco, S.P.A.*, 714 F.2d 1110, 1116, 219 USPQ 185, 188 (Fed. Cir. 1983).

Moreover, the claims are not to be read in a vacuum; the limitations therein are to be interpreted in light of the specification giving them their broadest reasonable interpretation. *In re Marosi, Stabenow, Schwarzmann, Lank*, 710 F.2d 799, 218 USPQ 289, 292 (Fed. Cir. 1983). 35 U.S.C. §112, second paragraph requires only reasonable precision in delineating the bounds of the claimed invention. The claim language is satisfactory if it reasonably apprises those of skill in the art of the bounds of the claimed invention and is as precise as the subject matter permits. *Shatterproof Glass Corp. v. Libby-Owens Ford Co.*, 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir. 1985), *cert dismissed*, 106 S. Ct. 340 (1985):

> [i]t is not necessary that a claim recite each and every element needed for the practical utilization of the claimed subject matter (*Bendix Corp. v United States*, 600 F.2d 1364, 1369, 220 Ct. Cl. 507, 514, 204 USPQ 617, 621 (1979).

ANALYSIS

Applicant respectfully disagrees with the Examiner's assertion, at page, 3, lines 10-13, that:

> The terms "sub" and "low" are not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably

> apprised of the scope of the invention. It is not clear as to what Applicant considers a "sub" or "low" amount of nanoliter volume.

It is respectfully submitted that, in view of the specification, those of skill in the art would readily understand what is encompassed by an amount of material in the "sub to low" range. For example, the specification teaches, at p. 19, 2nd paragraph, that:

> The sample **arrays** are then analyzed by mass spectrometry to collect spectra data that is representative of the composition of the samples in the array. It is understood that the above methods provide processes that allow for rapidly dispensing definite and controlled volumes of analyte material. **In particular these processes allow for dispensing sub to low nanoliter volumes of fluid.** These low volume deposition techniques generate sample arrays well suited for analysis by mass spectrometry.

In addition, the specification teaches, at page 26, last paragraph, that:

> Mass spectra were also obtained from DNAs microdispensed into the wells of a silicon chip. Figure 7 shows a 12 x 12 mm silicon chip with 100 chemically etched wells; mask dimensions and etch time were set such that fustum (i.e., inverted flat top pyramidal) geometry wells with 800x800μm (top surface) and 100μm depth were obtained. Optionally, the wells can be roughed or pitted. As described above, the chip edge was aligned against a raised surface on the stage to define the x and y coordinate systems with respect to the capillary. (Alternatives include optical alignment, artificial intelligence pattern recognition routines, and dowel-pin based manual alignment). **Into each well was dispensed 20 droplets ($\sim$5 nL) of 3-HPA matrix solution without analyte**; for the 50% CH_3CN solution employed, evaporation times for each droplet were on the order of 5-10 seconds.

The specification, at p. 23, lines, 5-9, teaches that:

> Robot-driven serial and **parallel pL-nL dispensing tools** were used to generate 10-10^3 element DNA arrays on $<$1" square chips with flat or geometrically altered (e.g. with wells) surfaces for matrix assisted laser desorption ionization mass spectrometry analysis.

The specification, at p. 25, lines 2-5, teaches that:

> The capillary was then filled with matrix solution, again checked at the stroboscope, and then used to spot an array onto flat or pitted

surfaces. For reproducibility studies in different MS modes, **typically a 10x10 array of 0.2-20 nL droplets were dispensed.**

Accordingly, in view of the specification, it is respectfully submitted that those of skill in the art would readily understand that the phrase "sub to low" in the context of nanoliter volumes encompasses picoliter volumes, certainly volumes as low as 0.2 nL, up to low nanoliter volumes depending upon the number of droplets delivered. It is respectfully submitted that the terms sub and low with respect to nanoliter volumes reasonably apprise those of skill in the art of the bounds of the claimed invention and are as precise as the subject matter permits.

Nonetheless, while not conceding the propriety of the rejection, in the interest of advancing claims to issuance, claim 1 has been amended to recite that the volume is 0.2 to 20 nanoliters in lieu of "sub to low," thereby rendering this rejection moot.

THE REJECTION OF CLAIMS 1-6, 9-34, 40-51, 54-61, 63-69 and 87-93 UNDER 35 U.S.C. § 102(b)

Claims 1-6, 9-34, 40-51, 54-61, 63-69 and 87-93 are rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Zhang *et al.*, *J. Mass Spec. 30*:1768-1771 (1995). The Examiner alleges that "... fluid is dispensed from the vesicle at each location of the set for forming an array of fluid material (Fig. 1), which clearly shows the array wherein the spot-to-spot characteristics are reproducible (page 1769, 2nd column, 1st full paragraph)." This rejection is respectfully traversed.

RELEVANT LAW

Anticipation requires the disclosure of each element of the claim under consideration in a single prior art reference. *In re Spada*, 15 USPQ2d 1655 (Fed. Cir. 1990), *In re Bond*, 15 USPQ 1566 (Fed. Cir. 1990). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention". *In re Lang*, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). Moreover, it is incumbent on the Examiner to identify wherein each and every facet of the claimed invention is disclosed in the reference. *Lindemann Maschinen-fabrik Gmbh*

v. American Hoist and Derrick Co., 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984).

Disclosure of the cited reference

Zhang *et al.* discloses that compounds of interest can be present at attomole or femtomole levels in relatively large volumes of solutions that may contain high volumes of salt. Zhang *et al.* states that in order to "achieve high sensitivity" for such samples, a dilute sample should be concentrated to a small volume and "*spotted directly*" on to a target, and salts, which may interfere with MALDI, should be removed. Zhang *et al.* is directed to methods for removal of salts and describes use of C_{18}-packed fused-silica capillary as a multifunctional device for micro-concentration, desalting and matrix addition for the analysis of trace levels of peptides in samples containing physiological salts. Salts are washed with water and the peptides eluted with an organic phase that contains the MALDI matrix, at low flow rates, to achieve sample spot sizes of "about 5 nl."

Zhang *et al.* discloses the use of a sample preparation device that includes the fused-silica capillary packed with the C_{18} particles, a multi-port injector with a 10 μl loop, and syringes mounted on syringe pumps. Sample introduction into the capillaries was performed by direct injection through the 10 the 10 μl loop and loaded on to the C_{18}-packed capillary to remove the salt. The eluate was directly spotted onto a multi-well sample holder with a "volume of *about* 5 nl per fraction." Zhang *et al.* states that the desalting and concentration "increased the MALDI sensitivity for dilute peptide samples" and an increase in signal to noise ratio was observed for the desalted samples compared to the untreated samples. Zhang *et al.* states that the desalting procedure permits quantitative determination of peptide. Furthermore, Zhang *et al.* is concerned with sensitivity of MALDI achieved by desalting samples, not by deposition of defined and controlled volumes.

Zhang *et al.* does not disclose a method in which vesicles dispense "defined and controlled volumes" nor methods in which dispensing is achieved such vesicles do not touch the surface of the substrate.

The Zhang reference is directed to increasing MALDI-mass spectrometry sensitivity by removing salts and reducing sample volume. For example, page 1769, 1st column, 2nd paragraph, states:

> Removal of salts and reduction of the sample volume from a few microliters to about 5 nl produced a sample spot area of less than 0.3 mm^2. The desalting and concentration effect **dramatically increased the MALDI sensitivity** for dilute peptide samples even when present in high salt concentrations. (emphasis added)

Moreover, Applicant respectfully disagrees with the Examiner's assertion that:

> ... fluid is dispensed from the vesicle at each location of the set for forming an array of fluid material (Fig. 1), which clearly shows the array wherein the spot-to-spot characteristics are reproducible (page 1769, 2nd column, 1st full paragraph).

It is respectfully submitted that the only disclosure in Zhang *et al.* with respect to reproducibility is merely directed towards the quantitative reproducibility of the procedure for samples at various concentrations. For example, at page 1769, 2nd column, 1st full paragraph (cited by the Examiner), Zhang *et al.* discloses:

> The **quantitative reproducibility** of the procedure was measured using samples of substance P in a solution containing 0.2 M NaCl. A loading volume of 10 μl was used which contained 5 fmol of neurotensin as an internal standard. The results were obtained as a plot of the substance P/neurotensin ratio vs. substance P **concentration**. A linear relationship ($r^2 = 0.9975$) was achieved in the concentration range 10 fmol μl^{-1}-100 amol μl^{-1}. This range is **two orders of magnitude lower** than that which could be obtained from direct quantitative analysis of even purified salt-free samples. (emphasis added)

It is respectfully submitted that quantitative reproducibility over a concentration range 10 fmol μl^{-1}-100 amol μl^{-1} is merely related to the overall sensitivity of the methods with respect to detection limits, and teaches nothing related to the quality or characteristics of the mass spectra obtained from each spot on the array.

Analysis

The claims and differences from Zhang *et al.*

Claim 1 and claims dependent thereon

Independent claim 1 is directed to:

> A method for forming an array of a sample material on a surface of a substrate and analyzing the sample material in the resulting array, comprising:
>
> providing a vesicle that has an interior chamber containing a fluid comprising a solvent containing the sample material;
>
> disposing said vesicle adjacent to a first location on said surface of the substrate *without contacting the surface with the vesicle*;
>
> providing mechanical pressure to the interior of the vesicle to eject from said chamber a *defined and controlled 0.2 to 20 nanoliter volume* of the fluid to dispense said fluid at said first location of said surface of the substrate;
>
> moving said vesicle to each of a set of positions adjacent to the surface of the substrate, whereby a defined and controlled 0.2 to 20 nanoliter volume of fluid is dispensed at each location of said set forming an array of spots of sample material on the substrate such that spot-to-spot characteristics are reproducible in the array; and
>
> performing mass spectrometry analysis of the sample material at each location of the array, wherein mass spectra of the sample obtained from each spot are reproducible within the array of spots.

Claim 2 recites that the surface has wells; claim 3 recites that the sample contains matrix material. Claim 4 recites that the method of claim 3, further includes a step of waiting a predetermined period of time to allow the solvent containing the matrix material to evaporate on the surface of the substrate thereby depositing the matrix material on the surface. Other dependent claims of the substrate, dispensing device and other elements and parameters.

Zhang *et al.* does not disclose a method of depositing samples "without contacting the surface of a substrate." Zhang *et al.* states that sample is spotted onto a surface; there is no disclosure of deposition without contacting the surface. nor does it disclose a method in which defined and controlled volumes are deposited. Zhang *et al.* spots "about 5 nl fractions." Therefore, since Zhang *et al.* does not disclose every element of the method as claimed, Zhang *et al.* doe not anticipate claim 1 nor any claims dependent thereon.

Claim 5

Independent claim 5 is directed to the method similar to claim 1 where a defined and controlled volume of solvent containing matrix material is deposited on the surface and allowed to evaporate; and then a defined and controlled volume sample is deposited at each locus dissolving the matrix and forming a crystalline structure containing the matrix and analyte at each locus.

Zhang *et al.* does not disclose a method in which matrix material is first deposited and then allowed to evaporated, followed by deposition of analyte such that analyte-containing sample dissolves into the deposited matrix to form a crystalline structure. Thus, for the reasons set forth with respect to claim 1 and further, since Zhang *et al.* does not teach the steps of separately depositing matrix and analyte, Zhang *et al.* does not anticipate claims 5 and 6.

Claim 25 and claims dependent thereon

Independent claim 25 is directed to:

A method for analyzing a material, comprising:

providing a vesicle comprising a fluid containing the material in a solvent;

disposing said vesicle adjacent to a first location of a surface of a substrate without contacting the surface with the vesicle;

delivering a defined and controlled nanoliter volume of the fluid at the first location of said surface of the substrate;

moving said vesicle to a second position next to the first location on said surface of the substrate to dispense a defined and controlled nanoliter volume of said material along an array of locations on said substrate surface to form an array of the material such that spot-to-spot characteristics are reproducible in the array; and

performing mass spectrometry analysis for said material at each location of said array, wherein mass spectra of the sample obtained from each spot are reproducible within the array of spots.

As with claim 1 and dependents claim, Zhang *et al.* does not disclose a method of depositing samples "without contacting the surface of a substrate" nor does Zhang *et al.* disclose deposition of a defined and controlled volume. Therefore, Zhang *et al.* does not anticipate claim 25 or any claims dependent thereon.

Claims 31-34

Claims 31-34 are directed to the systems for forming an array of a sample material on surface of a substrate and for analyzing the array of sample material. The systems include a vesicle having a distal end suitable for carrying a nanoliter of fluid; a movable arm having a distal portion mounted to move the vesicle; a controller for moving the arm to dispose the vesicle adjacent to a first location on the surface of the substrate and for controlling the vesicle to provide a nanoliter volume of the fluid at the first location of the surface of the substrate; and a mass spectrometer for analyzing the material deposited on the surface of the substrate by generating a composition signal representative of the chemical composition of the material.

Zhang *et al.* does not disclose a system for forming an array of sample and analyzing the sample that includes a movable arm having a distal portion mounted to move the vesicle and a controller for moving the arm to dispose the vesicle adjacent to a first location on the surface of the substrate and for controlling the vesicle to provide a defined and controlled 0.2 to 20 nanoliter volume. Therefore Zhang *et al.* does not anticipate any of claims 31-34.

Claims 40-51 and 54-61 and 63-69

Claims 40-51 and 54-61 and 63-69 are directed to methods for dispensing nanoliter volumes of a material as an array onto the surface of a substrate, comprising the steps of: (a) providing an assembly having a plurality of vesicles arranged in the form of array for dispensing a liquid therefrom, wherein each vesicle has an interior chamber containing a fluid containing the material; (b) aligning the vesicles at a first set of locations adjacent to surface of a substrate without contacting the surface with the vesicle; (c) using mechanical pressure, controlling each of the chambers to eject a nanoliter volume of the fluid from each vesicle onto the surface of the substrate aligned with the vesicles; and (d) providing the resulting substrate with the array of material deposited thereon to

mass spectrometer for determining information representative of the composition of the deposited material.

As with the other independent claims dependent claims specify the composition of the material that is deposited, the types of vesicle and means for applying pressure, and additional method steps. Claims 91-93, specify that the mass spectrometry format is MALDI.

As discussed above with respect to claim 1, Zhang *et al.* does not describe a method in which defined and controlled volumes are dispensed nor a method in which the vesicle does not contact the surface of a the substrate. Further, Zhang *et al.* does not disclose a method that includes providing an assembly having a plurality of vesicles arranged in the form of array for dispensing a liquid therefrom, wherein each vesicle has an interior chamber containing a fluid containing the material. The device of Zhang *et al.* is used to spot a single locus on a sample holder. Therefore, Zhang *et al.* does not anticipate any of claims 40-51 and 54-61 and 63-69.

Therefore, since Zhang *et al.* does not disclose every element as claimed, it does not anticipate any of the pending claims.

THE REJECTION OF CLAIMS 70-86 AND 94 UNDER 35 U.S.C. § 103(a)

Claims 70-86 and 94 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Ershow *et al.*, which teaches a tool for dispensing 0.3 nanoliter volumes on the surface of a substrate, in view of Jespersen *et al.* or Li *et al.* because Ershow *et al.* teaches a pintool apparatus and Jespersen *et al.* or Li *et al.* teaches the use of mass spectrometric analysis of small volumes. The Examiner concludes that it would have been obvious to one of ordinary skill in the art to have provided the method and apparatus of applying nanoliter volumes as taught by Ershow *et al.* with a detection system, as taught by Jespersen *et al.* or Li *et al.* in order to provide a significant reduction of the sample volume needed and three orders of magnitude improvement in detection limits. This rejection is respectfully traversed.

Relevant law

In order to set forth a *prima facie* case of obviousness under 35 U.S.C. § 103: (1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (*ACS Hospital Systems, Inc. v. Montefiore Hospital*, 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed. Cir. 1984)) and (2) the combination of the cited references must actually teach or suggest the claimed subject matter. Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. *Ex parte Gerlach*, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art" *In re Keller*, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed subject matter, absent some teaching or suggestion supporting the combination (*ACS Hosp. Systems, Inc. v Montefiore Hosp.* 732 F.2d 1572, 1577. 221 USPQ 929, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" *W.L. Gore & Associates, Inc. v Garlock Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

The prior art must provide a motivation whereby one of ordinary skill in the art would have been led to do that which the applicant has done. *Stratoflex Inc.*

v Aeroquip Corp., 713 F.2d 1530, 1535, 218 USPQ 871, 876 (Fed. Cir. 1983). In addition, the mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 USPQ 1783 (Fed. Cir. 1992).

Also, it is impermissible to ignore the advantages, properties, utilities and unexpected results that flow from the claimed invention; they are part of the invention as a whole. *In re Sernaker*, 702 F.2d 989, 217 USPQ 1 (Fed. Cir. 1983). Unexpected properties must always be considered when determining obviousness. A compound's structure and properties are inseparable so that unexpected properties are part of the subject matter as a whole. *In re Papesh*, 315 F.2d 381, 137 USPQ 43 (CCPA 1963).

The claims

First it is noted that claims 87-91 depend from claim 1, which is directed to a method that employs a vesicle that has an interior chamber containing a fluid comprising a solvent containing the sample material, and claim 93 is dependent on claim 40, which is directed to a method that uses an assembly having a plurality of vesicles arranged in the form of array for dispensing a liquid therefrom, wherein each vesicle has an interior chamber containing a fluid containing the material. Claim 92 is directed to a system of claim 31, which was not rejected over these references. Hence these claims appear to be outside the scope of this rejection, which is directed to claims that employ pin assemblies having a plurality of

elongated vesicles arranged as an array for dispensing a liquid therefrom. Ershow *et al.*, as discussed below and previously, does not teach or suggest any method that employs a vesicle that has an interior chamber containing a fluid.

Independent claim 70 and its dependents are directed to methods for dispensing nanoliter volumes of a material as an array on the surface of a substrate and analyzing the material in the array, by:

(a) providing a pin assembly having a plurality of elongated vesicles arranged as an array for dispensing a liquid therefrom, wherein each vesicle comprises a solid shaft of material having an end for retaining a nanoliter volume of fluid;

(b) loading a nanoliter volume of fluid comprising a liquid material from a fluid source onto the end of the vesicles of the pin assembly;

(c) disposing the pin assembly to align the vesicles at a first set of locations adjacent to a surface of the substrate without contacting the surface with the vesicles;

(d) contacting the loaded fluid to the surface of the substrate aligned with the vesicles to deposit a defined and controlled 0.2 to 20 nanoliter volume at each location, whereby an array of spots of material on the surface of the substrate is formed, such that spot-to-spot characteristics are reproducible in the array; and

(e) analyzing the array of material on the surface of the substrate by mass spectrometry, wherein:

mass spectra of the material obtained from each spot are reproducible within the array of spots;

the substrate comprises matrix material;

the fluid comprises analyte material;

the fluid of analyte material at the end of the vesicles is contacted with the evaporated matrix material on the surface of the substrate to dissolve the matrix material with the analyte material and thereby deposit a mixture of matrix and analyte material.

Independent claim 78 is directed to a method for dispensing nanoliter volumes of a material as an array on the surface of a substrate and analyzing the material in the array, comprising the steps of:

(a) providing a pin assembly having a plurality of elongated vesicles arranged as an array for dispensing a liquid therefrom, wherein each vesicle comprises a solid shaft of material having an end for retaining a nanoliter volume of fluid;

(b) loading a nanoliter volume of fluid comprising a liquid material from a fluid source onto the end of the vesicles of the pin assembly;

(c) disposing the pin assembly to align the vesicles at a first set of locations adjacent to a surface of the substrate without contacting the surface with the vesicles;

(d) contacting the loaded fluid to the surface of the substrate aligned with the vesicles to deposit a defined and controlled 0.2 to 20 nanoliter volume at each location, whereby an array of spots of material on the surface of the substrate is formed, such that spot-to-spot characteristics are reproducible in the array; and

(e) analyzing the array of material on the surface of the substrate by mass spectrometry, where:

mass spectra of the material obtained from each spot are reproducible within the array of spots;

steps of (a) through (d) are first performed with the vesicles containing a fluid comprising analyte material; and

after a predetermined time during which the analyte material evaporates on the surface, steps (a) through (d) are repeated to deposit fluid containing matrix material such that the fluid of matrix material at the end of the vesicles is contacted with the evaporated analyte material on the surface of the substrate to dissolve the matrix material with the analyte material and thereby deposit a mixture of matrix and analyte material.

Thus, claim 70 is directed to a method in which a defined and controlled volume containing analyte is deposited at loci on a substrate that has been preloaded with matrix material at each locus. Claim 78 is directed a method in which a defined and controlled volume containing analyte is deposited on the substrate and allowed to evaporate followed by deposition of matrix.

Teachings of the cited references

Ershow *et al.*

Ershow *et al.* teaches a tool for transferring small volumes. Transfer is effected by using free surface end of a rodlike transferring element, which is maintained at essentially the dew point of the ambient air during the transfer. The

device can include a plate-like base to which are affixed a plurality of rods such that the unfixed butt ends of the rods are coplanar. The device also includes a means for maintaining the temperature of the unfixed butt ends of the rods essentially equal to the dew point of the ambient air during transfer of the aqueous substance.

Ershow *et al.* does not teach or suggest that volumes dispensed must be controlled and defined to produce a substrate with uniform spots, nor does it teach or suggest deposition of the defined and controlled volume without touching the pin tool to the surface. Ershow *et al.* is directed to solving problems associated with evaporation and viscosity increases. Ershow does not teach a method in which analyte is deposited onto matrix nor a method in which first analyte is deposited followed by deposition of matrix. Ershow does not teach a method that includes a step of analyzing the array of material on the surface of the substrate by mass spectrometry. Ershow does not disclose or suggest that dispensing a plurality of defined and controlled volumes would result in an array of spots, where mass spectra of the material obtained from each spot are reproducible within the array of spots. As shown in the DECLARATION of record, mass spectra of the material obtained from each spot are reproducible within the array of spots.

As discussed below, neither Jespersen *et al.* nor Li *et al.* cures the deficiencies in the teachings of Ershow *et al.*

Jespersen *et al.*

Jespersen *et al.* teaches the application of "picolitre" vials for reducing sample matrix volume for analysis of crude samples. Jespersen *et al.* teaches

MALDI analysis of a small volume as way to lower the detection limit of MALDI analysis. Jespersen *et al.* does not teach or suggest depositing defined and controlled volumes to produce arrays of such sample material such that spot-to-spot characteristics are uniform. Jespersen *et al.* does not teach or suggest that such uniformity is a requisite for obtaining uniform spectra in high throughput formats.

Li *et al.*

Li *et al.* describes the analysis of single mammalian cell lysates using MALDI mass spectrometry. In the method described by Li *et al.*, a fused silica capillary is used to apply a volume of sample, which is determined by the length of the sample plug in the capillary, to a single location on the center of a MALDI probe precoated with a thin layer of matrix. The sample spot is always placed near the center of the probe using a microscope to position the sample spot in or near the center of the probe (p. 11662, rt. col., first full paragraph, lines 11-17).

Li *et al.* states that as little as 20 pl of sample solution can be accurately delivered on the matrix layer producing an ~100 μm diameter spot on the probe surface. The laser desorption beam is stated to be a 50 x 180 μm oval that is prealigned with the center of the probe surface. Although Li *et al.* states that the idea of microspot MALDI is to reduce the sample presentation surface with respect to the laser desorption site and ion acceptance volume in the mass spectrometer to improve sampling efficiency, Li *et al.* teaches that by rotating the sample probe, MALDI mass spectra are recorded from different areas in the sample spot.

Li *et al.* describes the method as a mass spectrometric approach for highly sensitive detection of small-volume protein samples such as are obtained from a lysate of a single cell. It is stated in Li *et al.* that the system provides attomole sensitivity for peptides and that further improvements of the sensitivity should be possible. The focus of the experiment described in Li *et al.* was to investigate the sensitivity of mass spectrometric analysis of the small volumes of material obtain from a single cell. It is concluded that the work described in Li *et al.* illustrates that

loading and analyzing a small-volume single cell is feasible by the microspot MALDI technique, and that the spectra and results shown are very reproducible for repeated preparations. Li *et al.* further states that the addition of several other features to this technique including quantitation, affinity separation, nanoliter or picoliter chemical and enzymatic reactions and tandem MS should further expand the usefulness of the mass spectrometric approach.

Neither Jespersen *et al.* nor Li *et al.* teaches or suggests the increase in reproducibility from spot-to-spot by delivery or deposition of defined and controlled volumes results in uniform spectra as shown in the DECLARATION of record. None of the cited references teaches a method in which analyte is deposited onto matrix nor a method in which analyte is deposited followed by deposition of matrix.

Analysis

There is no motivation to have combined Ershow *et al.* with Jesperson *et al.* or Li *et al.*

Ershow *et al.* is directed to devices for microdipensing very small volumes of aqueous solutions, particularly, solutions of oligonucleotides, in arrays; there is no mention of mass spectrometric analysis.

Jesperson *et al.* is directed to MALDI analysis of and the use of small volume as way to lower the detection limit of MALDI analysis. Li *et al.* is describes the analysis of single mammalian cell lysates using MALDI mass spectrometry. In the method described by Li *et al.*, a fused silica capillary is used to apply a volume of sample, which is determined by the length of the sample plug in the capillary, to a single location on the center of a MALDI probe precoated with a thin layer of matrix. The sample spot is always placed near the center of the probe using a microscope to position the sample spot in or near the center of the probe (p. 11662, rt. col., first full paragraph, lines 11-17).

Each of Jesperson *et al.* and Li *et al.* is directed to analysis of a small volume, but neither suggests an array in which defined and controlled volumes are deposited nor arrays of spots with uniform spot-to-spot characteristics. Jesperson

et al. is concerned with the detection limits of proteins of MALDI-MS and Li *et al.* is directed to single cell analyses and detection of small volume samples.

In fact, Li *et al.* may be viewed as, in effect, teaching away from such. Li *et al.* states that only a single sample spot is applied to a probe and is always placed near the center of the probe. Li *et al.* does not teach or even suggest depositing more than a single spot of sample solution to anywhere except the center of a MALDI probe surface. Although Li *et al.* mentions that the spectra and results are reproducible for repeated single preparations, each being individually analyzed as a single spot on the probe, it does not teach or suggest a substrate containing an array of spots of sample solution wherein **the characteristics of each spot are highly reproducible within the array** which are particularly well suited for use for large-scale, high-throughput mass spectrometric analysis in applications, *e.g.*, DNA diagnostics, where accuracy and reproducibility are critical. Similarly, Jesperson *et al.* is directed to the use of picoliter vials, but does not teach or suggest deposition of a defined and controlled volume of material into an array of such vials, nor results derived therefrom.

Nowhere in Li *et al.* or Jespersen is an array of spots containing matrix material in an amount resulting from deposition of the material onto a substrate in defined and controlled sub- to low-nanoliter volumes taught or suggested where **the characteristics of each spot are highly reproducible within the array**. Therefore, the combination of teachings does not result in the instantly claimed methods and systems.

Thus, each of Li *et al.* and Jesperson are directed to methods for increasing the sensitivity of MALDI analysis. Ershow is directed to a method for dispensing solutions such that evaporation is avoided. Absent the instant application, there is no suggestion in Ershow that would have lead the ordinarily skilled artisan to combine its teachings with those of Jespersen *et al.* or Li *et al.*

The combination of teachings does not teach or suggest delivery of defined and controlled volumes to an array nor the results achieved

Notwithstanding the fact that there is no motivation to have combined Ershow *et al.* with Li *et al.* or Jespersen *et al.*, the combination of these references does not result in the instantly claimed methods.

It is respectfully submitted that Ershow *et al.* and Jespersen *et al.* or Li *et al.*, whether alone or in combination, does not result in the instantly claimed methods. Ershow *et al.* does not teach or suggest the use of mass spectrometry for analysis of the materials deposited on a substrate by its device. Jespersen *et al.* is directed to an assessment of the detection limits of proteins by MALDI by delivering small volumes into picoliter vials to assess the detection limits, and Li *et al.* is directed to analysis of crude lysates on a probe. Li *et al.* is not directed to analysis of a plurality of samples, and Jespersen *et al.* provides an approach to reducing sample/matrix volumes. Neither Jespersen *et al.* nor Li *et al.* suggests analysis of samples deposited by the device and methods of Ershow *et al.* by mass spectrometry. Furthermore none of the cited references teaches or suggests a method in which analyte is deposited onto matrix nor a method in which first analyte is deposited followed by deposition of matrix.

Therefore, neither Jespersen *et al.* nor Li *et al.* cures the deficiencies in the teachings of Ershow *et al.* and the combination of teachings of the cited references does not result in the instantly claimed methods.

The prior art must provide a motivation whereby one of ordinary skill in the art would have been led to do that which the applicant has done. *Stratoflex Inc. v Aeroquip Corp.*, 713 F.2d 1530, 1535, 218 USPQ 871, 876 (Fed. Cir. 1983).

Furthermore, the combination of teachings of the references does not suggest the results that are achieved by depositing defined and controlled nanoliter volumes and analyzing the resulting arrays by mass spectrometry. As noted by the Examiner, Jespersen *et al.* suggests that decreasing the volume for analysis increases detection limits. This may be correct, but these are not the results achieved by the instantly claimed methods and systems. As shown in the DECLARATION, and discussed in previous responses, deposition of defined and

controlled nanoliter and smaller volumes to produce substrates with uniform spots and analysis by mass spectrometry results in uniform mass spectra among the samples in the array. The DECLARATION demonstrates that the sample array formed by nanoliter volume dispensing methods having the above-described properties contributes to the shortened spectrum acquisition time (Declaration, paragraph 9), increased detection sensitivity (Declaration, paragraph 10) and makes sample handling far more routine and amenable to automation (Declaration, paragraph 11). When the miniaturized sample dispensing methods were used in dispensing biological samples, *e.g.*, dispensing samples generated in a temperature-cycled PROBE reaction, highly sensitive and accurate analysis could be achieved. This permits use of such substrates in high throughput mass spectrometry formats. None of the cited references, singly or in any combination thereof teaches or suggests these results.

* * *

In view of the above remarks and the amendments and remarks of record, consideration and allowance of the application are respectfully requested.

Respectfully submitted,
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